Stable Isotope Ecology of the Red-Necked Wallaby (*Macropus rufogriseus*): Clarifying Species-Specific Responses to Climate and Geographic Variables

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BRIEF. Here, we aim to assess how *Macropus rufogriseus* tracks Australian climates via stable isotopes from tooth enamel.

ABSTRACT. Oxygen and carbon isotopes found in tooth enamel have been commonly used to infer past climates. However, relationships between climate variables and isotope data have rarely been thoroughly examined at the species level. Here, we clarify how the extant red-necked wallaby *Macropus rufogriseus* records known climate and/or geographic information (e.g., mean annual temperature, relative humidity, latitude, and δ^{18}Oprecipitation) in its teeth. Our results demonstrate that mean annual temperatures are the best predictors of δ^{13}C and δ^{18}O values in red-necked wallaby tooth enamel, although relative humidity has a weak but significant relationship with δ^{18}O values. Further, the red-necked wallaby is sensitive to changes in temperature and/or aridity and useful to assessing historic and prehistoric climate change. Future work examining how different species of macropods record their environment and climate is necessary to elucidating the mechanisms behind disparate relationships between climate and geochemical data.

INTRODUCTION.

Australia is currently experiencing significant climate change with temperature rise of approximately 0.8 to 2.8° C expected by 2050 [1]. Understanding how plants and animals respond to climate change, including Australian ecosystems, is the focus of much research [e.g., 2-4]. In addition to ecological studies of living plants and animals, the fossil record can provide insights regarding long term responses to climate change [e.g., 5-6]. For example, geochemical tools including stable isotope analyses of tissues (e.g., tooth enamel and/or bone) can record climate and dietary information, providing a means of determining both how climates have changed and how animals and plants have responded to those changes. However, in order to properly assess geochemical data it is first necessary to understand how various animals are "recording" climate data in their tissues.

A series of researchers have paved the way for using macropod (kangaroos and wallabies) stable isotopes (from tooth enamel and bone) for climate and dietary reconstructions [7-10]. Specifically, oxygen isotopes from bone phosphate are correlated with relative humidity [7], with greater δ^{18}O values occurring in more arid environments. Similar correlations are observed in tooth enamel; however, Murphy and co-authors [8] suggest there is an additional interaction between relative humidity and mean annual temperature. Further, stable nitrogen isotopes from bone collagen are correlated with mean annual rainfall [10]; however, as nitrogen is not always present in fossils (as nitrogen is more likely to decay and/or become diagenetically altered, as compared to highly inorganic tissues like bone and tooth enamel) studies of bone and tooth enamel are more relevant for deep time assessments of mammalian responses to climate change. While geochemical analyses of modern kangaroos and wallabies have allowed for further deep time climate assessments [e.g., 11-12], it is also necessary to move beyond genus level assessments and better understand how and potentially why different species of kangaroos and wallabies record climate information differently.

Kangaroos and wallabies can have highly variable life history and physiological traits [13]. For example, the majority of macropods reproduce aseasonally and the majority are capable of some form of embryonic diapause (i.e., a reproductive strategy where the embryo remains in a state of dormancy until conditions for reproduction are ideal) [14]. However, some wallabies such as the Tammar wallaby (*Macropus eugenii*) are known to reproduce seasonally [13]. Similarly, the red-necked wallaby (*Macropus rufogriseus*) is documented to reproduce seasonally in Tasmania and less so, on the mainland (excluding periods of extreme drought, or shortly after) [13, 15-16]. Further, kangaroos vary dramatically in their ability to tolerate and occupy different environments and climates with *M. rufogriseus* occupying cooler and typically wetter environments than the red kangaroo (*Macropus rufus*) and wallaroo (*Macropus robustus*) [8]. As physiology may further influence stable oxygen isotope values [17], a macropod's ability to concentrate their urine and even drink sea-water [13, 18-19] may further influence the δ^{18}O values of their tissues. Thus, while prior work has documented a strong relationship between δ^{18}O values and relative humidity [8], further work understanding species specific responses are necessary to better understand how geochemical proxies assess current and past climate change.

MATERIALS AND METHODS.

Stable isotopes.

Extant red-necked wallabies from throughout the majority of their geographic range were sampled from museum specimens from the Australian Museum and Museum Victoria Mammalogy collections. Last erupting molars (to reduce the potential influence of the mothers milk, as can be incorporated in earlier erupting teeth) were drilled using a carbide dental burr and Dremel® drill at low speed. Enamel powder was collected at respective museums and processed at Vanderbilt University. Specifically, the enamel was pretreated with hydrogen peroxide (30%) for 24 hours and acetic acid (1.0 N) for 18 hours to remove organics and secondary carbonates, respectively. Methods identical to that used by DeSantis *et al.* [5] were used here. Treated enamel powder (~1mg) was subsequently run on an in-line ISOCARB automatic sampler in the Department of Geological Sciences at the University of Florida (analytical precision is ±0.1‰, based on replicate analyses of samples and standards, i.e., NBS-19). Stable isotope data from the carbonate portion of tooth enamel hydroxylapatite were normalized to NBS-19 and are reported in conventional delta (δ) notation for carbon (δ^{13}C) and oxygen (δ^{18}O), where δ^{13}C (parts per mil, ‰) = ((Rsample/Rstandard)-1)*1000, and R = 13C/12C; and, δ^{18}O (parts per mil, ‰) = $((R_{sample}/R_{standard})-1)*1000$, and R = 18O/16O; and the standard is VPDB (Pee Dee Belemnite, Vienna Convention [20]). In addition to new data here acquired, we also include 29 samples from Murphy *et al.* [8] and analyze relationships between climate and geographic variables in all 53 samples. Data from Murphy *et al.* [8] were further analyzed at the species level (i.e., prior analyses focused on relationships between all kangaroos and various climate and geographic parameters) and are also included in the results section. Further, some $\delta^{18}O_{enamel}$ values are reported using (VSMOW‰, standard meteoric ocean water) from Murphy *et al.* [8]; however, as the standard conversion [21] did not identically replicate the conversion of prior data [8], all integrated analyses kept and compared all δ^{18}O data in reference to VPDB. Collectively, these data are from red-necked wallabies from a broad geographic range.

Climate data.

Geochemical data were compared to the temperature and precipitation data compiled from their respective areas. Specifically, climate data (i.e. mean annual temperature, °C) were gathered from climate stations most closely corresponding with the sites where specimens were collected via the Australian

Bureau of Meteorology website: http://www.bom.gov.au/climate/data/. Data for each station were then retrieved from the National Climatic Data Center and averaged by month collected between January 1, 1950 and December 31, 2009. Any months containing more than 5 days without reported data and any year without data for every month, was removed. The closest station to a given specimen's latitude and longitude, with at least 5 years of complete data was selected. Precipitation $\delta^{18}O$ (VSMOW‰) values were estimated using decimal degrees latitude and longitude coordinates and elevation (m) for each site via the online isotopes in precipitation calculator at: www.waterisotopes.org. Relative humidity data were acquired via the U.S. National Aeronautics and Space Administration (NASA) and Surface meteorology and Solar Energy (SSE) at the following website: http://en.openei.org/datasets/node/616. These data are regional averages of relative humidity at 10 meters above the surface of the earth and created on December 10, 2007 using data from July 1, 1983 to June 30, 2005. Annual relative humidity (RH) values for an individual specimens were estimated using the spatial analysis tool (extraction by point) in Arc GIS 10 and were compared to geochemical data.

Statistical analyses.

Linear regressions were used to assess relationships between geochemical data and climate variables using XLSTAT. To compare the strength of various models, we used the lowest Akaike's Information Criterion (AIC) value of a given model to indicate the best fit (compared to comparable models), as was done by Murphy and co-authors [8]. Specifically, we assess the following variables: relative humidity, mean annual temperature, and latitude. We also evaluated the relationship between enamel $\delta^{18}O$ values and modeled precipitation $\delta^{18}O$ values. *P*-values less than 0.05 are here considered significant.

RESULTS.

Stable oxygen isotopes.

Geochemical data of extant macropods from Murphy *et al.* [8] were reanalyzed at the species level and demonstrated varying relationships between $\delta^{18}O_{enamel}$(VSMOW‰) values and relative humidity, mean annual temperature, and $\delta^{18}O_{precipitation}$(VSMOW‰) values (Supplemental Table 1). While all kangaroos analyzed by Murphy and co-authors demonstrated a negative relationship between $\delta^{18}O_{enamel}$(VSMOW‰) and relative humidity, the strength of these relationships varied from R^2 values of 0.12 to 0.63 in *Macropus agilis* and *M. fuligionsus*, respectively. Further, relationships between $\delta^{18}O_{enamel}$(VSMOW‰) values and mean annual temperature were strongest and significant in *M. fuligionsus, M. giganteus,* and *M. rufogriseus*, specifically those macropods exposed to lower mean annual temperatures [8]. Red kangaroo *M. rufus* $\delta^{18}O_{enamel}$(VSMOW‰) values are significantly negatively correlated with mean annual temperature, the opposite of all other species except those present in the most arid and warmest areas (i.e., *M. agilis* and *M. robustus*).

When all $\delta^{18}O_{enamel}$ (VPDB‰) values from our data collection (n=24) and Murphy *et al.* [8] are combined, $\delta^{18}O_{enamel}$ (VPDB‰) values range from -5.2 to 2.5 (VPDB‰) with a mean value of -1.8 (standard deviation = 1.6). Although the standard deviation reduced from 1.8 to 1.6 when including all 53 samples, the mean and range remain identical between prior data [8] and all data collected to date, reported here. These data when compared to relative humidity data from NASA and SSE and mean annual temperature demonstrate a significant positive relationship with relative humidity, mean annual temperature, $\delta^{18}O_{precipitation}$ values, latitude, and combinations of these variables (Table 1). However, the model with the best fit is mean annual temperature, independently (Table 1). Also, it should be noted that the explanatory power of the relationship between $\delta^{18}O_{enamel}$ values and relative humidity dropped when using relative humidity data here included, in contrast to estimates from ANUClim 5.1 (Centre for Resource and Environmental Studies, Australian National University, Canberra; R^2=0.56 in Murphy *et al.* [8] and R^2=0.26 here, using the same 29 specimens from Murphy *et al.* [8], with *p*-values of <0.0001 and 0.005, respectively).

Table 1. Linear regression models between kangaroo $\delta^{18}O_{enamel}$(VPDB‰) and climate and/or geographic variables (*n*=53, including data from Murphy *et al.* [8]; D=AIC of the model minus the lowest AIC value per isotope model).

Isotope	Model	AIC	Δ	R^2	*p*-value
Oxygen	Relative Humidity (RH)	47.886	12.397	0.11	0.013
	Mean Annual Temperature (MAT)	35.489	0.000	0.30	<0.0001
	$\delta^{18}O$ (precipitation)	49.315	13.826	0.09	0.029
	Latitude	39.149	3.660	0.25	<0.001
	RH + MAT	36.043	0.554	0.32	<0.0001
	RH + $\delta^{18}O$ (precipitation)	44.609	9.120	0.20	0.004
	MAT + $\delta^{18}O$ (precipitation)	36.656	1.167	0.31	<0.0001
	RH + MAT + $\delta^{18}O$ (precipitation)	36.841	1.352	0.33	<0.001
Carbon	Relative Humidity (RH)	126.105	25.842	0.04	0.163
	Mean Annual Temperature (MAT)	100.263	0.000	0.41	<0.0001
	Latitude	102.207	1.944	0.39	<0.0001
	RH + MAT	101.971	1.708	0.41	<0.0001

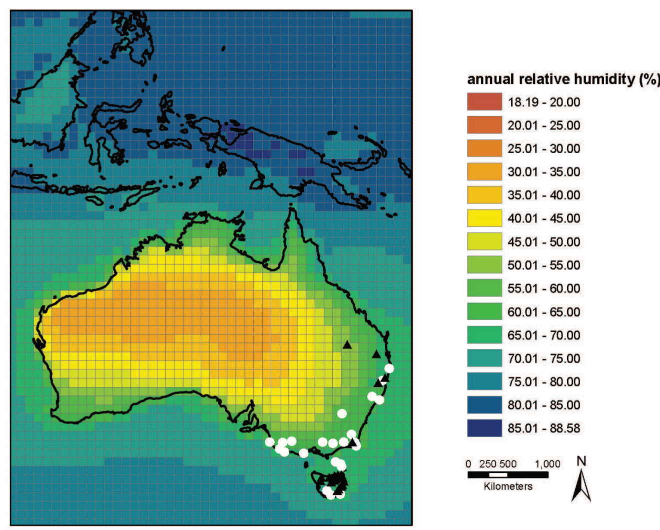

Figure 1. Location of all *Macropus rufogriseus* specimens sampled here (circles) and Murphy *et al.* [8] (triangles) in relation to mean annual relative humidity.

Stable carbon isotopes.

Carbon isotopes values (VPDB‰) range from -21‰ to -7.4‰ (total range of 13.6‰), with a mean $\delta^{13}C$ value of -16.7‰ (+/- 3.3 standard deviation, SD). Despite the additional sampling of 24 specimens (and increased longitudinal range of ~6 degrees), mean and range $\delta^{13}C$ values are highly consistent with prior analyses (mean from 29 samples from Murphy *et al* [8] = -17.4 +/- 3.1‰ SD, with a range from -21 to -8.6‰). Both latitude and mean annual temperature were significantly correlated with $\delta^{13}C$ values, with mean annual temperature yielding the best model fit (Table 1). Latitude and mean annual temperature are also significantly correlated (Pearson 0.70, *p*=<0.0001, R^2=0.49), as expected. Further, $\delta^{13}C$ and $\delta^{18}O$ values are significantly correlated (Pearson 0.52, *p*=<0.0001, R^2=0.26).

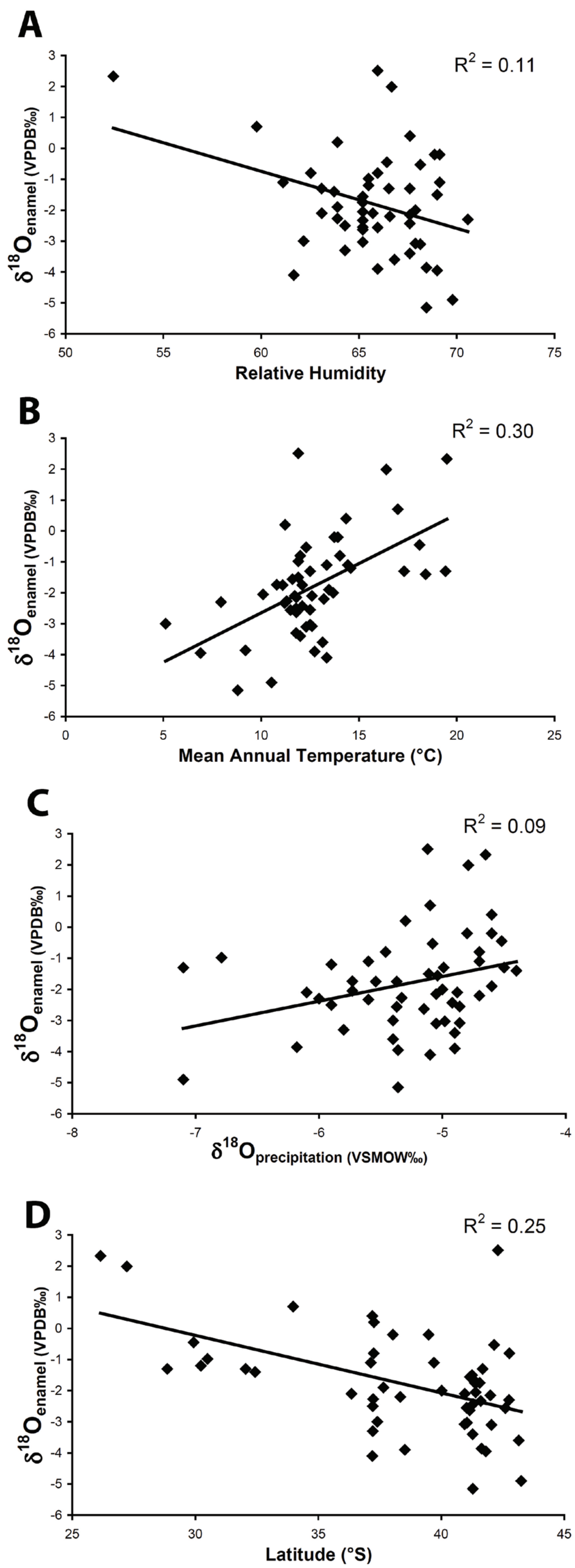

Figure 2. Bivariate plots of relative humidity (A), mean annual temperature (B), precipitation $\delta^{18}O$ (VSMOW‰) values (C), and latitude (D) versus enamel $\delta^{18}O$ (VPDB‰) values, of all *Macropus rufogriseus* examined here (including data from Murphy *et al.* [8]).

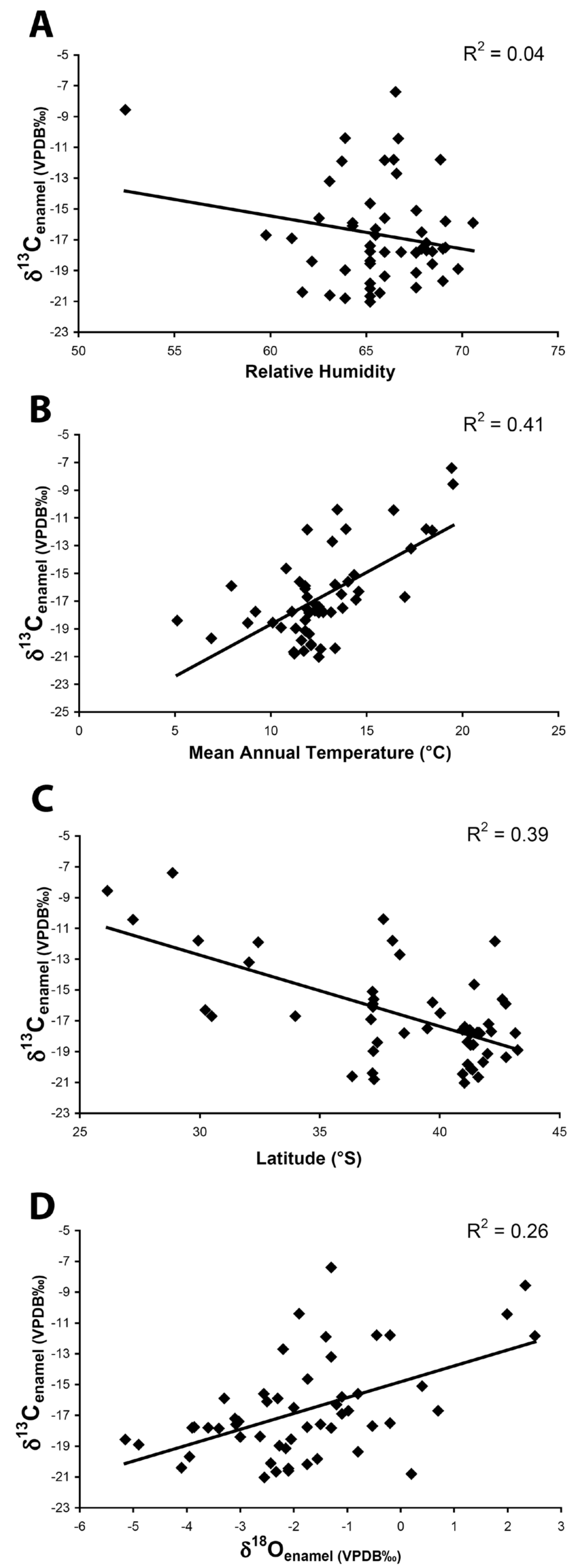

Figure 3. Bivariate plots of relative humidity (A), mean annual temperature (B), latitude (C), and tooth enamel $\delta^{18}O$ (VPDB‰) values (D) versus enamel $\delta^{13}C$ (VPDB‰) values of all *Macropus rufogriseus* examined here (including data from Murphy *et al.* [8]).

DISCUSSION.

Although prior work on the stable isotope ecology of the genus *Macropus* by Murphy and co-authors have shown relative humidity to be the best predictor of $\delta^{18}O_{enamel}$ values [8], this study added additional *M. rufogriseus* data from a broader geographic distribution than those already published and further demonstrated how specific species reflect known climate variables. Species level analysis of prior work [8] demonstrates that not all species of the genus *Macropus* track climate variables similarly (e.g., mean annual temperature). Here, mean annual temperature is a better predictor of $\delta^{18}O$ and $\delta^{13}C$ values, as compared to all other climate data analyzed. Although there is still a significant relationship between relative humidity and $\delta^{18}O$ values in *M. rufogriseus*, this relationship is fairly weak (R^2=0.11); thus, mean annual temperature likely has a greater influence on $\delta^{18}O$ and $\delta^{13}C_{enamel}$ values. This is reasonable as temperature affects the plants that grow in certain areas (e.g., C_3 vs. C_4 flora) and therefore what specific food sources are available to the inhabitants [22-23]. As the $\delta^{13}C$ values of plants and latitude are highly correlated both today and in the past [22-24], the strong correlation between *M. rufogriseus* enamel $\delta^{13}C$ values and latitude further suggests that *M. rufogriseus* is a generalist and consuming forage in proportion to its availability. While, carbon isotope values are highly correlated with latitude and mean annual temperature, future work is needed to decipher the textural properties of consumed food to assess the relative proportion of grass vs. browse throughout their range.

There is a less perceivable relationship between $\delta^{18}O_{precipitation}$ and $\delta^{18}O_{enamel}$ values, suggesting that kangaroos are not directly tracking rainfall water. Instead, *M. rufogriseus* $\delta^{18}O_{enamel}$ values are likely a reflection of the plant water (which is further affected by temperature) they consume rather than free water sources in the local area. Thus, we provide additional evidence that kangaroos are sensitive to aridity [7-8,10] and should be classified as "evaporation sensitive" taxa (keeping with the classifications of Levin and co-authors [25]).

Collectively, stable isotopes from tooth enamel of *M. rufogriseus* are useful predictors of past temperatures. Additionally, this study emphasizes the importance of examining the relationships between tooth geochemistry and climate data at the species level. *Macropus rufogriseus* are found in the southeastern and eastern regions of Australia where the climate is cooler and tends to be wetter; therefore, relationships described here are likely indicative of how more temperate kangaroos record climate data in their teeth. Responses recorded here may be different for other species of *Macropus* found at lower latitudes and in warmer, drier climates [8, 13]. Future research aims to examine how different species of kangaroos and wallabies track climate data in areas where ranges overlap.

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SUPPORTING INFORMATION.

Table S1. Relationship between $\delta^{18}O_{enamel}$(VSMOW‰) and climate variables including relative humidity (RH, %), mean annual temperature (MT, °C), and $\delta^{18}O_{precipitation}$($\delta^{18}$OP, VSMOW‰) of seven species of extant kangaroos (all primary data are from Murphy *et al.* [8]; *n*=694, including all tooth positions).

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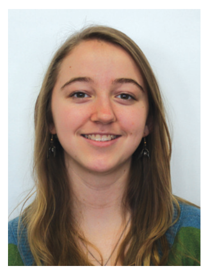

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